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ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 135
29 January 1954

PITOCIN RESTORATION OF RENAL FUNCTIONS TO PRE-NEUROHYPOPHYSECTOMY LEVELS

The Effect of Administering Neurohypophysial Extraction Products Upon the Reduced Renal Functions Associated with Neurohypophysectomy*

*Subtask under Environmental Physiology, AMRL Project No.6-64-12-028, Subtasks, Neuro-Endocrine and Renal and Body Fluid Responses to Environmental Variables.

RESEARCH AND DEVELOPMENT DIVISION
OFFICE OF THE SURGEON GENERAL

Army Medical Research Lab. Project No. 6-64-12-028 Report No. 135

PITOCIN RESTORATION OF RENAL FUNCTIONS TO PRE-NEUROHYPOPHYSECTOMY LEVELS; The Effect of Administering Neurohypophysial Extraction Products Upon the Reduced Renal Functions Associated with Neurohypophysectomy

Truman W. Desmukrun, Allen D. Keller, Abner H. Levkoff and Roy M. Purser, Jr.

19 pp & ii 1 illus 29 January 1954 UNCLASSIFIED

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1. Neurohypophysectomy 2. Physiological role for oxytocic principle 3. Diabetes insipidus 4. Neurohypophysial renal functions

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The Effect of Administering Neurohypophysial Extraction Products Upon the Reduced Renal Functions Associated with Neurohypophysectomy*

by

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Physiology Department
ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY
29 January 1954

*Subtask under Environmental Physiology, AMRL Project No.6-64-12-028, Subtasks, Neuro-Endocrine and Renal and Body Fluid Responses to Environmental Variables.

Report No. 135
Project No. 6-64-12-028
Subtasks AMRL S-4 and S-5
MEDEA

29 January 1954

ABSTRACT

PITOCIN RESTORATION OF RENAL FUNCTIONS TO PRE-NEUROHYPOPHYSECTOMY LEVELS

The Effect of Administering Neurohypophysial Extraction Products Upon the Reduced Renal Functions Associated with Neurohypophysectomy.

OBJECT

To determine if the drastic reduction in glomerular filtration rate, renal plasma flow and tubular maxima associated with neurohypophysectomy are due to a deficiency in a neurohypophysial endocrine principle.

RESULTS

Administration of pituitrin and pitocin elevated the reduced renal functions associated with neurohypophysectomy to preoperative levels whereas administration of pitressin had no effect on these functions.

CONCLUSION

The reduction in renal function associated with neurohypophysectomy is due to a deficiency in the oxytocic principle which is normally elaborated by the neurohypophysis. This finding affords a physiological explanation for the occurrence of this principle in the male as well as the female.

RECOMMENDATION

The anatomical basis for the selective precipitation of physiological deficiencies in the active principles found in the two fractions (pitressin and pitocin) of the total extract of the neurohypophysis should be further investigated.

Submitted by:

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PITOCIN RESTORATION OF RENAL FUNCTIONS TO PRE-NEUROHYPOPHYSECTOMY LEVELS

The Effect of Administering Neurohypophysial Extraction Products Upon the Reduced Renal Functions Associated with Neurohypophysectomy.

I. INTRODUCTION

It is established that permanent reductions in glomerular filtration rate, renal plasma flow and tubular maxima follow hypophysectomy in the dog (1). It is generally accepted that depression of renal functions in such a preparation is the result of an adenohypophysial, rather than a neurohypophysial deficiency because, 1) the depressed functions are present in the hypophysectomized preparation which does not exhibit a diabetes insipidus, 2) no reductions of renal functions were observed in the original 5 hypothalamic puncture diabetes insipidus dogs studied (1), and 3) administration of "growth" (2, 3) and "adrenocorticotrophic" (4) extraction products have been reported to elevate renal functions to or toward prehypophysectomy levels.

More recently comparable reductions of renal functions have been observed following a neurohypophysectomy procedure which precipitated a well defined diabetes insipidus without associated obvious adenohypophysial involvement (5). This observation immediately suggested that the depression in renal activity in such preparations was due to an uncomplicated neurohypophysial deficiency and accordingly raised the question as to whether neurohypophysial substitution therapy would or would not have an elevating effect on the renal functions alluded to above.

It is the purpose of this report to describe the results of administering neurohypophysial extraction products on the renal activity of neurohypophysectomized preparations exhibiting, 1) a well defined diabetes insipidus, 2) permanent and marked reductions of certain renal functions, and 3) no obvious adenohypophysial deficiencies.

II. EXPERIMENTAL

A. Animals

Eleven adult, mongrel, female dogs, ranging in weight from 10 to 15 kgs were used in this investigation. The animals were maintained on a constant daily food intake of 10 gms of raw horse meat and 10 gms of Purina Laboratory Chow per kg of body weight. Water was provided ad libitum.

B. Neurohypophysectomy*

Under a barbiturate anesthesia the hypophysial area was exposed by the Bailey and Bremer modification of Cushing's subtemporal approach. The stalk was first cut completely across with scissors; a ball-electrode was then applied against the cut surface of the stalk, which remained attached to the hypothalamus, and a coagulating current applied (Bovie Electrosurgical Unit). This procedure assures by direct visibility, the complete destruction of the proximal portion of the pars nervosa (infundibulum) and separation of its remaining distal portion (infundibular process) from any anatomical continuity with the hypothalamus. In addition the latter procedure coagulates a portion of the ventral tuberal and ventral anterior hypothalamus, thus encroaching upon the area of the hypothalamus which, in common with the pars nervosa upon extraction, yields pituitrin and possesses a characteristic stainability and vascularity (8). Also, it is this area of the hypothalamus where Scharrer's secretory neurons are predominately located (9). Secretory neurons have been described in the infundibulum (10) but not in the infundibular process, yet a considerably greater yield of extracted pituitrin is derived from the infundibular process than from the anterior hypothalamus (11).

In the experience of this laboratory it has been necessary to impinge upon the ventral portion of the anterior hypothalamus in addition to removal or isolation of the pars nervosa in order to achieve a functional neurohypophysectomy as evidenced by, 1) a well defined diabetes insipidus (7), 2) a 50 per cent reduction in renal function (5), and 3) a measurably increased susceptibility to hemorrhage (12).

*It is appreciated that anatomically the term neurohypophysis is used in various ways. Most often it is used synonymously with pars nervosa (6). The usage in this and related reports is according to the following schemata which reflect, 1) this laboratory's experience in producing experimental neurohypophysial deficiencies, 2) reports in the literature describing the tissue from which pituitrin has been extracted, and 3) the suspicion that even though animals exhibit a compensated (clinical) permanent diabetes insipidus they may not be totally neurohypophysectomized (7).

NEUROHYPOPHYSIS

A. Pars Nervosa (hypophysial component).
   1. Infundibulum (stalk component).
   2. Infundibular process (posterior lobe component)

B. Pars Hypothalamus (the characteristic vascularity or secretory neurons in the central nervous system whichever, if either or both, proves to be the relevant anatomical characteristic to be correlated with neurohypophysial function and extraction of pituitrin potential).
   1. Ventral Tuberal and Ventral Anterior Hypothalamus (tangential or supra-optic nuclei area).
   2. Other areas in C.N.S. (Para-ventricular nuclei and/or locus caeruleus, etc.)

LEGEND FOR FIGURE 1

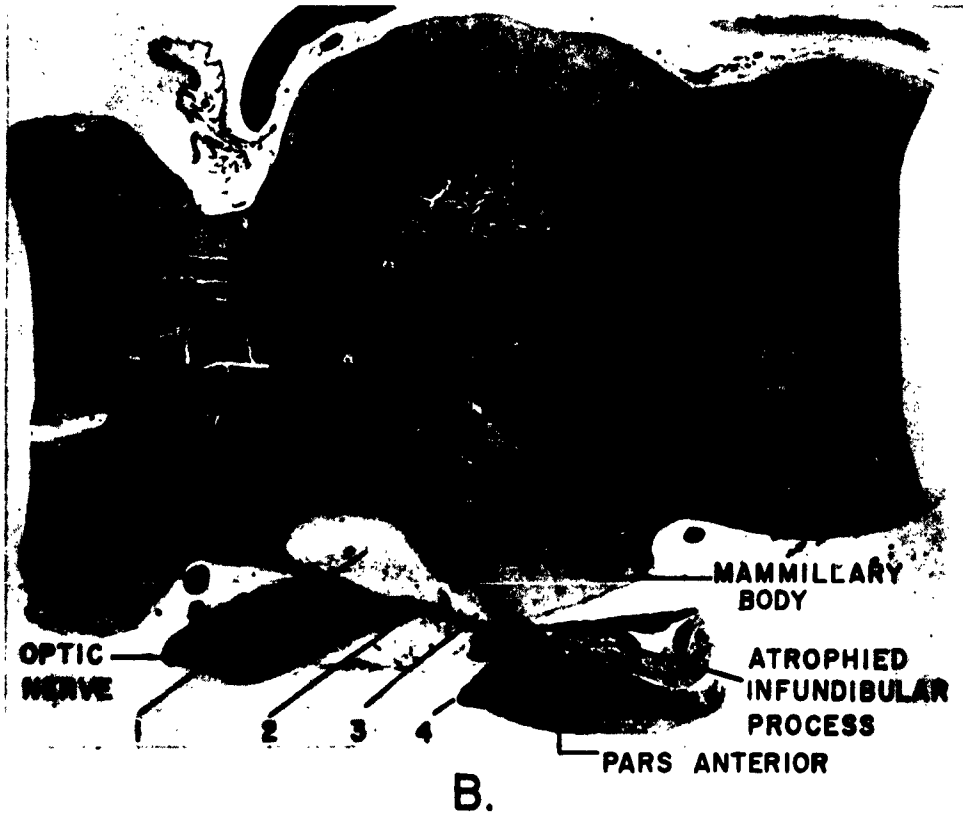
A. A photograph of a sagittal section taken from the Pal-Weigert series on Dog # 202. This section is from a somewhat lateral position such that the pars anterior is shown without the posterior lobe being in the field.

B. A photograph of a sagittal section taken from the Maximow series on Dog # 202. This section is cut through the hypophysis such that the shrunken and somewhat hollowed infundibular process is readily evident. Note the straight line of the scissor cut through the proximal tip of the pars anterior as indicated by the arrow.

Note in both photographs the complete absence of, 1) the ventral portion of the anterior hypothalamus, including the optic chiasm, 2) the tuberal component of the hypothalamus, and 3) the pituitary stalk (infundibulum and pars tuberalis).

DOG 202

A.



B.

1. VENTRAL ANTERIOR HYPOTHALAMIC COMPONENT OF TISSUE DEFECT.
2. VENTRAL TUBERAL HYPOTHALAMIC COMPONENT OF TISSUE DEFECT.
3. HYPOPHYSIAL STALK COMPONENT OF TISSUE DEFECT.
4. SCISSOR CUT.

The gland distal to the scissor cut (pars anterior, remnant of pars tuberalis and posterior lobe) was completely shielded from the coagulating current by virtue of the design and insulation of the electrode. The isolated adenohypophysis remained in its usual position in the pituitary fossa and from visual inspection at the time of closure did not appear to be disturbed.

The anatomical delimitation of a neurohypophysectomy as described above is illustrated in Figure 1 wherein photographs of 2 sections taken from the series on Dog # 202 are reproduced.

C. Laboratory Procedures

1. Renal Clearances and Tubular Maxima. - All experiments on renal functions were performed on unanesthetized animals 24 hours after the last feeding.

Glomerular filtration rate and renal plasma flow, as measured by exogenous creatinine and PAH clearances, as well as Tm PAH (tubular maximum for para-aminohippurate) were determined on 2 or more occasions previous to operation and again at intervals after the onset of permanent diabetes insipidus. Creatinine was determined by the method of Bonsnes and Taussky (13) and PAH by the method of Smith et al. (14).

Three 10-minute urine samples, with blood drawn at the mid-point of each period, were collected for the clearance determinations followed by 3 such periods for measuring Tm PAH. For the clearance determinations, creatinine and PAH were injected subcutaneously or constantly infused intravenously in amounts calculated to maintain constant serum levels of 20 mg per cent and 2 to 3 mg per cent respectively. For Tm determinations, a constant PAH serum level of 40 to 50 mg per cent was obtained by injection of a primer and infusion of an appropriate sustaining solution.

2. Administration of Neurohypophysial Extraction Products.

a. Pituitrin.

1) Aqueous Extract. - An aqueous preparation of whole gland extract, pituitrin, (Philadelphia Ampoule Company) was administered to diabetes insipidus dogs exhibiting reduced renal functions, in total doses ranging from 5 to 10 I.U. (International

Units). On a given experimental day, glomerular filtration rate and either renal plasma flow or tubular excretory maximum were determined immediately before administering pituitrin. Following these control periods, the total dose of pituitrin was given in 2 or more injections either subcutaneously or intramuscularly and so timed as to avoid a transitory rise in blood pressure during the time that the renal function measurements were being repeated.

In 4 of the experiments, blood pressures by direct arterial needle were measured with the Sanborn electromanometer before and 35 to 120 minutes after the injection of pituitrin.

2) Lyophilized Pituitrin. - A suspension of lyophilized pituitrin in peanut oil was administered subcutaneously immediately after 3 control clearance periods. One and one half hours later the clearances were again measured. The exact potency of these lyophilized preparations was not determined.

b. Pitressin

1) Pitressin Tannate in Oil. - Pitressin tannate (Parke, Davis) in oil was administered subcutaneously in doses of 5 pressor units every 48 hours. This amount was more than sufficient to return a diabetes insipidus dog's daily fluid exchange to the preoperative status. After several days of controlling fluid exchange with pitressin, the renal functions were again assessed. To insure an adequate urine flow when urine flow was restricted by pitressin, a mild osmotic diuresis was induced by the infusion of a 10 per cent mannitol solution at the rate of 1 cc per minute. It has been ascertained that such an infusion of mannitol has no effect upon the renal functions under investigation in the untreated diabetes insipidus dog.

2) Aqueous Pitressin. - In a limited number of experiments aqueous pitressin (Parke, Davis) was administered either subcutaneously or by constant infusion. Generally, on the day of the experiment, pitressin was administered subcutaneously in 1 or 2 injections in dosages ranging from 5 to 10 pressor units. Except when pitressin was constantly infused, which was at the rate of 5 to 20 milliunits per minute, the second injection was given at the beginning of the equilibration period previous to the Tm determination.

c. Pitocin

1) Aqueous Pitocin. - Commercial oxytocic extract (Parke, Davis pitocin) was administered either subcutaneously

or by constant infusion before and after which renal functions were determined. Two to 7.5 I. U. were injected subcutaneously in 2 injections as described for the pituitrin experiments. When pitocin was constantly infused following the pre-therapy control periods the animals received 0. 04 I. U. per minute for the remaining time of the experiment.

2) Lyophilized Pitocin. - In one instance a diabetes insipidus dog received a single injection of lyophilized aqueous pitocin suspended in peanut oil, immediately after ascertaining its renal clearance values. These determinations were repeated 1.5 hours, 24 hours and 1 week after the single injection.

d. Oxytocin. - The most highly purified extract of oxytocin available, prepared by du Vigneaud (15), was administered to 2 diabetes insipidus dogs. On the basis of a previous assay, 1 oxytocic unit of this extract was injected intravenously immediately after 3 pre-therapy clearance periods. This injection was followed by constant infusion of 0. 04 oxytocic units per minute before and during the remaining 6 urine collection periods.

The exact degree of oxytocic activity of this extract was not determined at the time it was used, but had been assayed by du Vigneaud some weeks previously. At that time it contained 5 oxytocic units per cc (16).

III. RESULTS

A. Attainment of Neurohypophysectomy

The neurohypophysectomized animals recovered uneventfully from the anesthesia and required no special nursing or therapeutic care other than keeping water continuously available.

1. Diabetes Insipidus. - On recovery from anesthesia the animals exhibited an immediate, well defined, diabetes insipidus which persisted indefinitely except for the characteristic and predictable transient disappearance of a few days duration, usually from the 6th to the 10th day after operation.

Table 1 contains representative 24-hour fluid intake and urine output volumes and urine specific gravities for each animal before operation and during permanent diabetes insipidus. Note that all 11 operated dogs showed a striking increase in the water ingested and urine excreted with a markedly decreased urine specific gravity.

TABLE 1
REPRESENTATIVE FLUID EXCHANGE VOLUMES
BEFORE AND AFTER OPERATION

Dog Number	UNOPERATED			DIABETES INSIPIDUS		
	Water Intake cc/24 hrs	Urine Output cc/24 hrs	Urine Sp.Gr.	Water Intake cc/24 hrs	Urine Output cc/24 hrs	Urine Sp.Gr.
99 (14 Kg)	410	173	1.054	4428	3517	1.004
100 (14 Kg)	390	306	1.045	3856	3521	1.007
128 (14 Kg)	400	220	1.055	3234	2739	1.005
130 (15 Kg)	454	286	1.057	4768	4362	1.005
153 (15 Kg)	380	280	1.045	2298	2022	1.003
202 (14 Kg)	540	420	1.053	5305	4403	1.001
204 (12 Kg)	500	280	1.045	3778	3441	1.004
205 (10 Kg)	360	140	1.045	3465	2958	1.004
206 (10 Kg)	380	200	1.040	2622	2435	1.005
222 (12 Kg)	320	160	1.050	3135	2854	1.005
245 (14 Kg)	300	202	1.050	3491	3174	1.004

2. Reduced Renal Functions. - Glomerular filtration rate, renal plasma flow and tubular excretory maximum data on 11 dogs are presented in Table 2. Each value is representative of several such determinations both before operation and after the onset of permanent diabetes insipidus. A marked reduction of these renal functions was encountered without exception in the entire series and persisted to time of autopsy, as long as 2.5 years postoperatively. Note that the reduction in these functions following neurohypophysectomy is approximately 50 per cent, as originally described by Handley and Keller (5).

3. Absence of Obvious Adenohypophysial Dysfunctions. - The animals exhibited no other obvious functional deficits except partial or occasionally total blindness depending upon the extent to which the optic chiasm and/or the optic tracts were coagulated.

TABLE 2

REDUCED RENAL FUNCTIONS ASSOCIATED WITH
A NEAR-TOTAL NEUROHYPOPHYSECTOMY

Dog Number	UNOPERATED			DIABETES INSIPIDUS		
	G.F.R.* cc/Min	R P.F.** cc/Min	Tm PAH*** mg/Min	G.F.R. cc/Min	R.P.F. cc/Min	Tm PAH mg/Min
99	45.6	118.3	17.8	26.8	85.8	6.5
100	62.2	163.4	14.5	32.7	96.6	8.3
128	57.3	154.9	-	34.4	90.1	-
130	68.3	182.3	-	42.6	132.6	-
153	39.4	115.0	-	20.2	70.9	-
202	47.0	145.1	15.0	30.2	86.0	9.8
204	60.0	195.1	17.8	28.9	106.1	6.9
205	42.3	116.1	10.4	29.9	82.8	5.7
206	48.3	124.6	16.4	29.9	97.2	8.8
222	39.4	114.8	12.8	21.3	61.6	5.5
245	49.8	120.1	-	32.2	86.0	-

* G.F.R. - Glomerular filtration rate.

** R.P.F. - Renal plasma flow.

*** Tm PAH - Tubular excretory maximum.

a. Insulin Tolerance Within or Near Normal Range. - Eight of the animals were subjected to a series of insulin tolerance tests, the results of which are presented in Table 3. Seven of the 8 animals showed a detectable decrease in insulin tolerance; 3 animals tolerated only one half unit, 3 tolerated only one fourth unit and one (# 245) tolerated only one tenth unit per kg body weight. These are measurable reductions in tolerance from the 1 unit per kg predictably tolerated by the mongrel dog (17). However, in magnitude such reductions are neglibible when it is remembered that 1/40th to 1/100th of a unit of insulin per kg elicits major convulsions in near-total hypophysectomized animals, resulting in the necessity of glucose therapy for survival.

TABLE 3

INSULIN TOLERANCE TESTS IN EIGHT DIABETES
INSIPIDUS AND TWO HYPOPHYSECTOMIZED DOGS

Dog Number	Insulin Administered (Units per Kg)		
	Test A	Test B	Test C
100	1/4	1/2	1*
153	1/4	1/2	1
202	1/4	1/2	1*
204	1/4	1/2*	
205	1/4	1/2*	
206	1/4	1/2	1*
222	1/4	1/2*	
245	1/10	1/4*	
198**	1/40	(Hypoglycemic Death)	
201**	1/40	(Hypoglycemic Death)	

* Glucose Therapy

** Hypophysectomized

b. Presence or Absence of Sexual Regression. - The change in coat to a soft, infantile texture which characterizes the hypophysectomized animal was not seen in any of the diabetes insipidus dogs in the series. Sexual regression was in evidence in some of the preparations and not in others (see data in Table 4). Some of the animals periodically exhibited signs of estrus with vaginal bleeding and swelling of the external genitalia. One dog (#153) was bred and subsequently whelped a litter of 5 normal appearing pups. The pups died in a matter of a few days apparently because of inability to obtain sufficient milk from the mother. The mammary glands were developed and the pups suckled normally.

Gross and/or macroscopic post-mortem examinations of 10 of the animals are summarized in Table 4*. Five of these had normal appearing genitalia with no indications of atrophy. In these animals inspection of the ovaries revealed that all stages of ovum development were clearly distinguishable including corpora lutea. The genitalia of 3 of the remaining animals examined were somewhat smaller than one might expect but were not markedly decreased in size. Histological examination of these ovaries revealed the presence of all stages of ovum development except corpora lutea. Whereas the genital tracts of one dog (#222) appeared normal, the cystic ovaries of this animal found at necropsy were typical of those often associated with senility.

Such indefinite anatomical indications of sexual hypofunction might reflect either partial sexual regression resulting directly from the experimental lesion or early stages of senility since 2 of these particular animals (#99 and #222) were known to be several years old.

c. Absence of Adrenal Atrophy. - Gross and histological examinations of the 10 animals autopsied revealed no evidence of adrenal cortical atrophy (see Table 4).

4. Neurohypophysial Tissue Defect at Necropsy. - The results of macroscopic inspection of the area of the tissue defect in the dogs which came to autopsy are summarized in Table 4. Photographs of representative sections from the series on one of the dogs (#202) are reproduced in Figure 1.

* Post-mortem examinations were made independently by F. A. Miller, Captain, M. C.

TABLE 4

MACROSCOPIC NECROPSY DATA

Dog Number	Neurohypophysis				Adenohypophysis		Adrenal Cortices	Genital Tracts and Ovaries
	Pars Nervosa		Hypothalamus					
	Infundibulum	Infundibular Process	Ventral Tuberal	Ventral Anterior	Pars Tuberalis	Infarction of Pars Anterior		
99	Absent	Shrunken	Moderate Infarction	Moderate Infarction	Absent	Trace	Normal	Non-Functional*
100	Absent	Hollowed	Absent	Absent	Absent	Moderate	Normal	Functional
128	Absent	Hollowed	Absent	Absent	Absent	Moderate	Normal	Functional
130	Absent	Shrunken	Absent	Absent	Absent	Trace	Normal	---
153	Absent	Shrunken	Absent	Absent	Absent	Trace	Possibly Hypertrophied	Functional (whelped pups)
202	Absent	Hollowed	Absent	Absent	Absent	Trace	Normal	Non-Functional
205	Absent	Hollowed	Absent	Absent	Absent	Moderate	Normal	Functional
206	Absent	Hollowed	Absent	Intact	Absent	Trace	Normal	Functional
222	Absent	Hollowed	Absent	Absent	Absent	Considerable	Normal	Non-Functional (senile appearing)
245	Absent	Shrunken	Absent	Moderate Infarction	Absent	Considerable	Normal	Non-Functional

*Non-functional designates ovaries which do not show maturation compatible with ovulation.

B. Effect of the Administration of Neurohypophysial Products Upon Renal Functions.

1. Pituitrin

a. Aqueous Extract. - The effect of administering the posterior lobe total extract upon the reduced renal functions of 6 neurohypophysectomized animals is presented in Table 5. A complete or near complete restoration of the reduced clearances was observed in all 6 dogs. Although pituitrin therapy consistently and markedly elevated the tubular excretory maximum for PAH, this function was not always completely restored to preoperative levels.

b. Lyophilized Pituitrin. - The administration of lyophilized pituitrin to 2 diabetes insipidus animals was found to elevate the reduced clearances to pre-neurohypophysectomy levels. Four animals were subjected to arterial puncture blood pressure measurements before and at intervals (35, 60, 80 and 120 minutes) after comparable doses of pituitrin. A detectable elevation of blood

pressure was found to occur in several experiments 35 to 60 minutes after administration of pituitrin. However, such transitory elevations in blood pressure appeared to bear no relationship to the increase in renal functions. Such a supposition arises from the fact that 80 to 100 minutes after the injection of pituitrin, during which time the renal functions were elevated, blood pressures were not found to be elevated from the pre-therapy values.

TABLE 5

PITUITRIN (AQUEOUS) THERAPY
DIABETES INSIPIDUS DOGS

Dog Number	Renal Functions	Preoperative Values	Permanent D.I. Pre-Therapy	Pituitrin Therapy
202 (14 Kg)	G.F.R.*	47.0	27.7	47.1
	R.P.F.*	145.1	82.8	127.6
	Tm PAH**	15.0	8.1	10.3
204 (12 Kg)	G.F.R.	60.0	28.9	50.0
	R.P.F.	195.1	106.1	184.2
	Tm PAH	17.8	6.9	12.7
205 (10 Kg)	G.F.R.	42.3	29.9	41.9
	R.P.F.	116.1	82.8	115.9
	Tm PAH	10.4	5.7	10.0
206 (10 Kg)	G.F.R.	48.3	29.9	50.3
	R.P.F.	124.6	94.2	124.0
	Tm PAH	16.4	8.8	12.3
222 (12 Kg)	G.F.R.	39.4	24.1	36.0
	R.P.F.	114.8	60.7	75.9
	Tm PAH	12.8	5.6	9.3
245 (14 Kg)	G.F.R.	49.0	31.0	67.0
	R.P.F.	120.0	89.0	145.0
	Tm PAH	-	-	-

* cc/minute
**mg/minute

2. Pitressin

a. Pitressin Tannate in Oil. - Representative renal function values of 5 diabetes insipidus dogs on pitressin tannate (oil) therapy are tabulated in Table 6. It was routinely observed that administration of 5 pressor units of pitressin in oil every 48 hours was sufficient to completely abolish the polyuria and polydipsia of the diabetes insipidus syndrome. However, it is apparent from the data presented that such a therapy regime had no obvious effect upon the reduced filtration rate, renal plasma flow and Tm PAH.

TABLE 6

PITRESSIN (OIL) THERAPY
DIABETES INSIPIDUS DOGS

Dog Number	Renal Functions	Preoperative Values	Permanent Diabetes Insipidus	Pitressin in Oil Therapy
99 (14 Kg)	G.F.R.*	45.6	26.8	29.3
	R.P.F.*	118.3	85.8	85.1
	Tm PAH**	17.8	6.5	9.4
202 (14 Kg)	G.F.R.	47.0	30.2	33.0
	R.P.F.	145.1	86.0	97.0
	Tm PAH	15.0	9.8	9.7
204 (12 Kg)	G.F.R.	60.0	28.9	29.3
	R.P.F.	195.1	106.1	87.5
	Tm PAH	17.8	6.9	6.5
206 (10 Kg)	G.F.R.	48.3	29.9	32.8
	R.P.F.	124.6	97.2	107.5
	Tm PAH	16.4	8.8	9.0
222 (12 Kg)	G.F.R.	39.4	21.3	23.0
	R.P.F.	114.8	61.8	53.3
	Tm PAH	12.8	5.5	4.2

* cc/minute
** mg/minute

b. Aqueous Extract. - Data from short-duration experiments in which aqueous pitressin was administered are presented in Table 7. Relatively large doses of aqueous pitressin administered on the day of the experiment had no clear-cut effect upon the reduced renal functions of 4 neurohypophysectomized animals. A very slight elevating effect was observed in some experiments in which very large doses of pitressin were administered which, it is felt, could be attributed to pitocin contamination of the pitressin.

TABLE 7

PITRESSIN (AQUEOUS) THERAPY
DIABETES INSIPIDUS DOGS

Dog Number	Renal Functions	Permanent Diabetes Insipidus	Aqueous Pitressin Therapy
205 (10 Kg)	G.F.R.*	28.0	25.9
	R.P.F.*	82.8	90.1
	Tm PAH**	4.5	5.5
(222) (12 Kg)	G.F.R.	27.2	30.1
	R.P.F.	61.5	66.5
	Tm PAH	5.6	6.4
130 (15 Kg)	G.F.R.	53.6	53.2
	R.P.F	148.1	140.7
	Tm PAH	11.0	10.9
100 (14 Kg)	G.F.R.	42.5	42.9
	R.P.F.	115.8	122.6
	Tm PAH	7.8	7.9

* cc/minute
** mg/minute

3. Pitocin

a. Aqueous Extract. - Data on the effect of pitocin administration upon the renal functions of 6 diabetes insipidus dogs are presented in Table 8. Pitocin was found to be just as effective in restoring the reduced clearances and Tm PAH as was pituitrin.

Isolated experiments in which a greater lapse of time (1.5 to 2 hours) was allowed between the administration of the oxytocic extract and the clearance periods resulted in more consistent and complete restoration of the reduced functions than the data presented in the table indicate.

TABLE 8

PITOCIN (AQUEOUS) THERAPY
DIABETES INSIPIDUS DOGS

Dog Number	Renal Functions	Preoperative Values	Permanent Diabetes Insipidus	Pitocin Therapy
202 (14 Kg)	G.F.R.*	47.0	27.7	51.7
	R.P.F.*	145.1	82.8	153.6
	Tm PAH**	15.0	8.1	13.5
204 (12 Kg)	G.F.R.	60.0	28.9	49.0
	R.P.F.	195.1	106.1	162.6
	Tm PAH	17.8	6.9	10.3
205 (10 Kg)	G.F.R.	42.3	29.9	45.0
	R.P.F.	116.1	82.8	104.4
	Tm PAH	10.4	5.7	5.9
206 (10 Kg)	G.F.R.	48.[illegible]	29.9	50.8
	R.P.F.	124.6	94.0	128.6
	Tm PAH	16.4	8.8	10.5
222 (12 Kg)	G.F.R.	39.4	24.1	40.0
	R.P.F.	114.8	60.7	86.6
	Tm PAH	12.8	5.6	8.6
245 (14 Kg)	G.F.R.	49.0	31.0	61.0
	R.P.F.	120.0	89.0	144.0

* cc/minute

** mg/minute

b. Lyophilized Pitocin. - One animal (#245) whose creatinine and PAH clearances had been assessed the previous day was given a single intramuscular injection of lyophilized pitocin suspended in peanut oil. Two hours after the injection the renal clearances were found to be restored to pre-diabetic levels. Twenty-four hours later, with no further therapy during the interim, the clearances were still elevated to pre-neurohypophysectomy levels; whereas 1 week later they were again reduced to half.

4. Oxytocin. - A highly purified extract of the oxytocic fraction of the posterior lobe extract, prepared by du Vigneaud, (op. cit.) completely restored renal clearances and markedly elevated the Tm PAH of 2 dogs.

IV. DISCUSSION

A. Extirpation Deficit -- Successful Replacement Therapy

The effective restoration to normal of the reduced renal functions associated with neurohypophysectomy by administration of pituitrin affords evidence that the reduction in renal clearances is directly due to a deficiency in a principle normally supplied by the neurohypophysis. This is a straight-forward example of an extirpation deficit which is remedied by replacement therapy. The finding that the replacement principle is contained in a highly purified fraction of the total extract of the neurohypophysis renders the evidence reasonably conclusive.

The possibility seems remote that the reduced clearances are due to a selective involvement of the adenohypophysis, i.e., equivalent to the sexual hypofunction which frequently obtains following a hypothalamic lesion without other adenohypophysial deficits being present as obtained in the series of dogs in this report (see Table 4) (18, 19). If a selective dysfunction of a particular cellular constituent of the adenohypophysis were responsible there would be the same incidence and magnitude of lowering in clearances following a series of hypophysial stalk sections. This is the case with sex atrophy but decidedly not so with renal clearances. The precipitation of a 50 per cent reduction in renal clearances clearly correlates with encroachment of the tissue defect upon the hypothalamic component of the neurohypophysis rather than with the macroscopic encroachment upon, or infarction of, the adenohypophysis. Thus, sectioning the pituitary stalk does not cause a lowering of renal clearances nor does this procedure precipitate diabetes insipidus; a mild encroachment upon the ventral hypothalamus following a stalk section precipitates diabetes insipidus without associated lowering of renal clearances, and a moderate encroachment upon the hypothalamus subsequent to the sectioning of the stalk predictably precipitates the 50 per cent decrease in renal clearances as well as diabetes insipidus.

In addition, if the reduced renal clearances in the neurohypophysectomized dog were the result of a selective "neighboring" in-

volvement of the adenohypophysis, the successful elevation of these functions to pre-neurohypophysectomy levels by pitocin could only be explained on the basis of pitocin being contaminated with the effective adenohypophysial replacement principle, namely, a "growth" (2) or "adrenotrophic" (4) principle. This seems improbable because there is little or no likelihood that pitressin would be free of such contamination if pitocin were so contaminated and the molecular weights of the adenohypophysial principles are many times greater than those of the posterior lobe principles; accordingly, there would be greater proneness for adenohypophysial extractions to be contaminated with the neurohypophysial principles than vice versa. In fact, it is known that certain growth (20) and adrenotrophic (4, 21) extraction products are contaminated with assayable amounts of posterior lobe principles.

There is also the possibility, which seems remote, that renal clearances per se are relatively labile and any deviation from an even physiological keel causes a non-specific drastic reduction in these functions. If this were the cause of lowered clearances in the neurohypophysectomized dog, then one would expect that controlling the diabetes insipidus with pitressin would return physiological stability and also the clearances. Also, the successful reversal of the clearances by pitocin would then have to be explained on the basis of a purely coincidental pharmacodynamic property of this extraction product.

B. A Physiological Role for the Oxytocic Principle

If the interpretation of the foregoing outlined physiological role for the oxytocic principle is correct, then here is an explanation for its existence in the male of the species as well as in the female. Previously, the only suggestion for a physiological role for this chemical factor was centered around its "uterine stimulating" and "milk let-down" pharmacodynamic properties. In the latter connection it is suspected that the failure of the neurohypophysectomized dog to raise its pups was directly due to a deficiency in "milk let-down" ability.

The fact that normal parturition occurs in the presence of well defined neurohypophysial deficiencies adds to one's suspicion that the neurohypophysial principles are not totally lacking in the type of neurohypophysectomized preparations described above and elsewhere (5, 7, 12). If this is not so then it must be concluded that the "uterine stimulating" property of pitocin does not have the physiological importance previously projected, namely, that its presence is essential for the initiation of parturition.

The finding of separate and distinct extirpation deficiencies which are selectively remedied by the pitressin and pitocin fractions of the total neurohypophysial extract may help to clarify the long-standing question as to whether the active constituents in the two fractions of pituitrin are separate and distinct endocrine principles or are merely chemical fragments broken off a parent substance during the extraction and separation procedures.

V. SUMMARY AND CONCLUSIONS

Glomerular filtration rate, renal plasma flow and tubular maxima are strikingly and predictably reduced by a neurohypophysectomy procedure in the dog which does not produce obvious adenohypophysial dysfunction.

Renal functions in such preparations are effectively restored to preoperative levels by administration of the total extract of the neurohypophysis (pituitrin) as well as by its oxytocic fraction (pitocin).

Administration of the pressor fraction of the total extract (pitressin) has no effect on renal functions in these neurohypophysectomized preparations.

The foregoing data are compatible with the interpretation that the lowered renal activity is due to an extirpation deficiency in the oxytocic neurohypophysial principle and that restoration of renal activity by pitocin to the pre-extirpation level constitutes direct replacement therapy.

If the foregoing interpretation is correct, the finding affords a physiological explanation for the occurrence of the oxytocic principle in the male as well as the female. Previously, the only suggestion for a physiological role for this chemical factor was centered around its demonstrated "uterine stimulating" and "milk let-down" pharmacodynamic properties.

VI. RECOMMENDATION

The anatomical basis for the selective precipitation of physiological deficiencies in the active principles found in the two fractions (pitressin and pitocin) of the total extract of the neurohypophysis should be further investigated.

VII. BIBLIOGRAPHY

1. White, H. L. and P. Heinbecker. Observations on creatinine and urea clearances, on responses to water ingestion and on concentrating power of kidneys in normal, diabetes insipidus and hypophysectomized dogs. Am. J. Physiol. 123: 566, 1938.

2. White, H. L., P. Heinbecker and D. Rolf. Enhancing effects of growth hormone on renal function. Am. J. Physiol. 157: 47, 1949.

3. De Bodo, R. C., I. L. Schwartz, J. Greenberg, M. Kurtz, D. P. Earle, Jr. and S. J. Farber. Effect of growth hormone on water metabolism in hypophysectomized dogs. Proc. Soc. Exper. Biol. & Med. 76: 612, 1951.

4. Earle, D. P., S. J. Farber, R. C. de Bodo, M. Kurtz and M. W. Sinkoff. Effects of ACTH, cortisone and hydrocortisone on renal functions of hypophysectomized dogs. Am. J. Physiol. 173, No. 2: 189, 1953.

5. Handley, C. A. and A. D. Keller. Changes in renal functions associated with diabetes insipidus precipitated by anterior hypothalamic lesions. Am. J. Physiol. 160, No. 2: 321, 1950.

6. Rioch, D. McK., G. B. Wislocki and J. L. O'Leary. A précis of preoptic, hypothalamic and hypophysial terminology with atlas. The Hypothalamus 20: 3-30, 1940.

7. a. Keller, A. D. Elimination of the pars nervosa without eliciting diabetes insipidus. Endocrinology, 30: 408-422, 1942.

   b. Levkoff, A. H., T. W. Demunbrun and A. D. Keller. The disparity between fluid intake and renal concentrating deficit in dogs with diabetes insipidus; polydipsia, independent of the renal concentrating deficit. Am. J. Physiol. 176, No. 1: 25-32, 1954.

8. Wislocki, G. B. and L. S. King. Hypophysis: permeability and blood supply. Am. J. Anat. 58: 421, 1936.

9. Scharrer, E. and B. Scharrer. Secretory cells within the hypothalamus. A. Research Nerv. & Ment. Dis. 20: 170, 1940.

10. Breckenridge, C. G. Intracellular granules in the hypothalamus and infundibulum of the dog. Fed. Proc. 6: 81, 1947.

11. a. Abel, J. J. Physiological, chemical and clinical studies on pituitary principles. Harvey Lecture 19: 154-211, 1923.

b. Trendelenburg, P. Anteil der hypophyse und des hypothalamus am experimentellen diabetes insipidus. Klin. Wchnschr. 7: 1679-1680, 1928.

12. a. Frieden, J. and A. D. Keller. Decreased resistance to hemorrhage in neurohypophysectomized dogs; evidence for a physiological role for pitressin as a vasopressor substance. AMRL Report # 129, 1953.

b. Frieden, J. and A. D. Keller. Decreased resistance to hemorrhage in neurohypophysectomized dogs. Circulation Research (in press).

13. Bonsnes, R. W. and H. H. Taussky. On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem. 158: 581, 1945.

14. Smith, H. W., N. Sinkelstein, L. Aliminosa, B. Crawford and N. Graber. The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. Clin. Invest. 24: 388, 1945.

15. Livermore, A. H. and V. du Vigneaud. Preparation of high potency oxytocic material by the use of counter current distribution. J. Biol. Chem. 180: 365, 1949.

16. Du Vigneaud, V. Personal Communication.

17. Keller, A. D., W. E. Lawrence and C. B. Blair. Effects of varying degrees of hypophysectomy in the dog. Arch. Path. 40: 289-308, 1945.

18. Breckenridge, C. G. and A. D. Keller. Retention of sex functions after isolation of the pars anterior by extirpation of the hypophysial stalk. Am. J. Physiol. 152: 591, 1948.

19. Keller, A. D. and J. W. Hamilton. Normal sex functions following section of the hypophysial stalk in the dog. Am. J. Physiol. 119: 349-350, 1937.

20. De Bodo, R. C., M. Kurtz, A. Ancowitz and S. P. Kiang. Anti-insulin and diabetogenic actions of purified anterior pituitary growth hormone. Am. J. Physiol. 163: 310-318, 1950.

21. Sprague, R. G. and M. H. Power. Observations on the metabolic effects of cortisone and ACTH in man. Pituitary Adrenal Function. A. A. A. S. p. 128-144, 1950.